STRATIFICATION AND DEFORMATION OF ARBACIA PUNCTULATA EGGS CENTRIFUGED IN CAFFEINE SOLUTIONS

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Introduction

During an investigation of the effect of methylated purines upon cellular behavior, the following problems arose: Does contact with the trimethylated purine, caffeine, which is known to influence cellular metabolism, affect the viscosity of the cytoplasm of the cell; and is there any evidence that this alkaloid influences the forces at the cell surfaces? Heilbrunn's informative studies (1926, 1928, 1943) on viscosity and surface forces, with reference to numerous chemical substances, did not deal adequately either with alkaloids as a group, or with caffeine in particular. Centrifugation followed by a study of the degree of granule stratification within the cytoplasm, together with changes in form of the cell itself, offered a satisfactory method of approach to these questions. From the experiments described below, it will be seen that caffeine does not alter the viscosity of the unfertilized egg but acts upon the membrane and cortical tension forces, thereby influencing cleavage in the fertilized egg. In the higher concentrations, even sperm entry is prevented by caffeine.

The temperature factor is not significant in the work reported here, since caffeinized eggs were centrifuged at the same time and temperature as control eggs from the same female. In this way, relative viscosity effects could be observed.

Methods and Materials

The author (1945, 1946a, b, 1946) has demonstrated by studies on O_2 consumption and comparative sensitivity of developmental stages that caffeine retards cleavage in Arbacia. In the present study, the unfertilized and fertilized eggs [unfertilized in sea water (SW), unfertilized in 0.10 per cent caffeine-in-sea-water (CSW); fertilized, i.e. normal egg (N ♀) × normal sperm (N ♂), in sea water, and fertilized N ♀ × N ♂ in 0.10 per cent caffeine-in-sea-water] were centrifuged at 10,000 × g for five, seven, and twelve minutes, and also at 3000 × g, 40 minutes after fertilization or at the equivalent time interval after shedding in the case of unfertilized eggs. This 40 minute period was chosen because that is when the viscosity of the protoplasm approaches the increased state typical at the time of cleavage. Comparable series employing other concentrations were also run.

Experiments were conducted at the temperature of running sea water. The appearance of uncentrifuged and centrifuged caffeinized eggs was compared with photomicrographs and descriptions by E. B. Harvey (1940). Differences in the degree of stratification (compactness) of the pigment granules and vacuoles and the height of the hyaloplasm zone after centrifugation were noted as evidence of

relative viscosities. To avoid error due to a time variable caused by the return of granules by Brownian movement, all photomicrographic records were made ten minutes after centrifugation.

Results and Discussion

Cleavage abnormalities in eggs centrifuged in CSW were no greater than those observed in the same concentration of CSW without centrifugation. There was no evidence that caffeine induced any primary change in viscosity which would prevent cleavage. Clearly defined effects were reproducible and similar in both unfertilized and fertilized eggs, but those in the former were more convincing because the normal viscosity changes during mitosis made it impossible to assume that controls and experimentals would be in exactly the same state.

Bank (1932), using *Arbacia punctulata,* reported stratification within the unfertilized eggs without centrifugation if they were held in 1 per cent caffeine for 48 hours. This is not surprising since caffeine was shown by the author (1945, 1948) to retard the O_2 uptake of the Arbacia cell. The facility with which the egg contents stratify due to such a factor as O_2 uptake cannot be determined by centrifugation. In the experiments described in this paper, an indication of a surface effect was the fact that Arbacia eggs cannot be fertilized when immersed in 1 per cent CSW. Both the eggs and sperm, however, survive for a considerable period in 1 per cent CSW, and the eggs can be fertilized and undergo partial development if transferred to sea water. Therefore, this concentration of caffeine does not destroy the internal physiological potentialities of these gametes with respect to fertilization. Over as long a period as 48 hours, the physical effect noted by Bank can be understood on the basis of the biochemical inhibition of cellular respiration, and/or as a surface effect, without assuming a primary viscosity change due directly to caffeine.

Among the results observed in the present series, the delay of deformation, reduction in actual fragmentation, and the sharper margins of the layers (apparent under the earlier conditions of the experiments) were the most readily distinguishable and clearly associated phenomena. De Vries (1947), in his studies on viscosity and tension at the surface in eggs of the fresh water snail, *Limnaea stagnalis* L., based the interpretation of his results primarily on the occurrence of vacuoles and granules in the hyaloplasm zone, as well as on the height of this zone. He pointed out that the height of the hyaloplasm zone depends on both viscosity and the degree of stretching, i.e. tension at the surface. Therefore, it occurred to the present writer that the closer packing of the pigment granules might be attributable to the fact that the pigment in the spherical cell had a shorter distance to fall than the pigment in the uncaffeinized normal cell, which is always elongated by centrifugal force to the degree applied in the experiments up to this time. It seemed desirable to eliminate the effect of the stretching factor and resulting deformation in order to clarify the significance of the degree of stratification in interpreting viscosity changes. Accordingly, unfertilized and fertilized eggs, both control and experimental, were centrifuged at only $3000 \times g$ for intervals varying from one-half to three minutes. These shorter centrifugations at $3000 \times g$ did not change the shape of the cells in either the control or the

caffeinized eggs, but did allow stratification. Therefore, comparisons of stratification could be made without the deformation factor.

In the absence of internal viscosity changes, the increased force required to break caffeinized eggs indicates a surface effect. Harvey (1931) estimated that the centrifugal force necessary to pull the Arbacia egg into two halves indicates that tension at the surface for a 25 per cent increase in area is less than 0.2 dyne per cm. with considerable variation in eggs. At 10,000 × g for 12 minutes, a count of five fields of each of the experimental caffeine series showed the percentage of breaking in the unfertilized eggs to be as follows: Controls in SW were 100 per cent broken; eggs in 0.02 per cent CSW, 12 per cent broken; in 0.10 per cent CSW, 0.50 per cent were broken; and in the 2.0 per cent CSW, only 0.08 per cent were broken although slight elongation did occur.

The apparent absence of any significant osmotic change (Cheney, 1948) as well as of demonstrable viscosity changes in the internal protoplasm, together with the delay in deformation reported here, would indicate that caffeine may initiate a change in the surface of the cell. Such an effect might involve both the membrane and the cortical protoplasm, which Harvey and Shapiro (1941) demonstrated to possess a considerably higher viscosity than the interior protoplasm in the eggs of *Arbacia punctulata* and *Asterias forbesii*.

Summary *

1. Caffeine does not change the existing viscosity state of the egg.

2. Egg fragmentation, under centrifugation, decreases with increased caffeine concentration.

3. The "apparent" effect of greater stratification of the granules in Arbacia eggs centrifuged in caffeine does not occur if the centrifugal force to which the eggs are subjected is sufficient to produce sedimentation but insufficient to cause deformation.

4. Evidence indicates that the delay of deformation in the caffeinized eggs, centrifuged at 10,000 × g or less, may be due to the action of caffeine (trimethylated purine) upon the total tension forces at the surface areas of both unfertilized and fertilized Arbacia eggs.

LITERATURE CITED

Bank, O., 1932. Stratification des œufs d'oursin sans centrifugation. *Compt. Rend. Soc. Biol.*, **110**: 389–390.

Cheney, R. H., 1945. The effects of caffeine on oxygen consumption and cell division in the fertilized egg of the sea urchin, Arbacia punctulata. *Jour. Gen. Physiol.*, **29**: 63–72.

Cheney, R. H., 1946a. Effect of caffeine concentration upon retardation of Arbacia development. *Biol. Bull.*, **91**: 226–227.

Cheney, R. H., 1946b. Sensitivity of Arbacia development to caffeine. *Anat. Record*, **96**: 547–548.

Cheney, R. H., 1948. Caffeine effects on fertilization and development in Arbacia punctulata. *Biol. Bull.*, **94**: 16–24.

* Centrifugation facilities and aids granted the author by Dr. E. B. Harvey during this study are deeply appreciated.

DeVries, G. A., 1947. The influence of lithium chloride and calcium chloride on viscosity and tension at the surface of uncleaved eggs of Limnaea stagnalis L. *Proc. Kon. Ned. Akad. v. Wetensch,* Amsterdam, **50**: 1335–1342.

Harvey, E. B., 1940. A comparison of the development of nucleate and non-nucleate eggs of Arbacia punctulata. *Biol. Bull.,* **79**: 166–187.

Harvey, E. N., 1931. The tension at the surface of marine eggs, especially those of the sea urchin, Arbacia punctulata. *Biol. Bull.,* **61**: 273–279.

Harvey, E. N., and H. Shapiro, 1941. The recovery period (relaxation) of marine eggs after deformation. *Jour. Cell. Comp. Physiol.,* **17**: 135–144.

Heilbrunn, L. V., 1926. The absolute viscosity of protoplasm. *Jour. Exp. Zool.,* **44**: 255–278.

Heilbrunn, L. V., 1928. The colloid chemistry of protoplasm. Monograph. Berlin.

Heilbrunn, L. V., 1943. An outline of general physiology. Ed. 2. Saunders Co.